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Compositions containing omega-3 fatty acid-containing oils and plant extracts

The present invention relates to compositions containing omega-3 fatty acid-containing oils and plant extracts and their use as a dietetic food product or medicament or pharmaceutical preparation having an increased bioavailability of the secondary plant ingredients contained in the preparations.

The term „secondary plant ingredients" comprises those ingredients of the plant, which are of no relevance as energy sources or structural substances. For the plant they serve for example as colorants, defensive agents or attractants. The number of secondary plant ingredients is estimated to be from 10,000 to 30,000 individual substances. With respect to their chemical structure or their biogenesis they can be classified into the following groups:

Polyphenols: This group comprises monobasic phenol carboxylic acids such as gentisinic acid, protocatechuic acid, gallic acid or caffeic acid as well as flavones such as kaempferol, quercetin, myricetin, isorhamnetin, naringenin, 6-prenylnaringenin, 8-prenylnaringenin, isoxanthohumol and their glycosides, chalcones such as xanthohumol, isoflavones such as daidzein and genistein, anthocyans such as pelargonidin, cyanidin, malvidin or delphinidin, tanning agents such as catechin and epicatechin as well as oligomers and polymers thereof.

Isoprenoids: This group comprises all compounds derived from isoprene such as monoterpenes such as thymol, menthol or carvone, diterpenes, triterpenes such as phytosterols (β-sitosterol, campesterol, stigmasterol), cardenolides, tetraterpenes such as carotenes.

Glucosinolates: Glucosinolates are β-S-glucosides of thiohydroxamic acids such as sinigrin, sinalbin or glucobrassicin.

Sulfides: Examples for members of this group comprise alliin and allicin.

The following plants having a high content of one of the groups of secondary plant ingredients mentioned above are mentioned as examples: Aesculus hippocastanum, Althaea, Allium cepa, Brassica nigra, Camellia sinensis, Carum carvi, Cimicifuga racemosa, Crataegus oxyacantha, Echinacea purpurea, Ginkgo biloba, Glycine max, Hedera helix, Humulus lupulus, Hypericum perforatum, Linum usitatissimum, Mentha piperita, Myrtus communis, Opuntia ficus-indica, Panax ginseng, Silybum marianum, Trifolium pratense, Vaccinium myrtillus, Vitex agnus-castus and Vitis vinifera.

The knowledge about health-promoting effects of secondary plant ingredients has strongly increased in the last years. Numerous epidemiologic or in vitro studies have shown the following effects: Anticancerogenic, antimicrobial, antioxidative, antithrombotic, immune-modulating, antiinflammatory, blood pressure-affecting, cholesterol-lowering, blood glucose-affecting and eupeptic (Watzl B., Leitzmann C. (1999) Bioaktive Substanzen in Lebensmitteln, Hippokrates Verlag).

The bioavailability data of different plant ingredients are still fragmentary. In case of an isolated administration a resorption of 24% was determined for the flavonoid quercetin, whereas the resorption of quercetin present in onions is 52%. (Hollmann P. C. H., de Vries J. H. M., van Leuwen S. D., Mengelers M. J. B., Katan M. B. (1995) Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. Am. J. Clin. Nutr. 62, 1276-1282; Hollmann P. C. H., Katan M. B. (1999) Dietary flavonoids: Intake, health effects and bioavailability. Food Chem. Toxicology 37, 937-942). 24 hours after oral uptake of flavanols such as epigallocatechin gallate, about one third of the dose was excreted with the faeces in an animal experiment (Sugunuma M., Okabe S., Oniyama M., Tada Y., Ito H., Fijiki H.

(1998) Wide distribution of 3H-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue, Carcinogenesis 19, 1771-1776). A bioavailability of 13 – 35 % was determined for isoflavonoids (Xu X., Harris K. S., Wang H. J., Murphy P. A., Hendrich S.(1995) Bioavailability of soybean isoflavones depends upon gut microflora in women. J. Nutr. 125, 2307-2315). In summary, to the extent known, the bioavailability of secondary plant ingredients is not very high.

The resorption is basically effected through the lipid double-layer of the mucosa of the gastrointestinal channel by passive diffusion, by carrier-mediated diffusion, by active transport and by pinocytosis/phagocytosis/persorption. The diffusion through the lipid matrix is quantitatively the most important factor. Therefore, the lipid solubility of the substance to be resorbed plays a dominant role. Highly polar substances such as amino acids, sugars and water-soluble vitamins are resorbed via an active transport. In this context, the transport protein p-glycoprotein in the intestinal mucosa, which serves to excrete undesired resorbed foreign substances back into the lumen of the gastrointestinal tract, is important. Likewise, a resorption is opposed by a possible metabolization by cytochrome P450 enzymes of the enterocytes.

In other words, the resorption system is designed such that nutrition components such as protein, fat and carbohydrates are resorbed primarily. However, vitamins and minerals are resorbed in addition to these nutrition components as well. Since the spectrum of polarities of the cited compounds ranges from apolar to polar, compounds have to be resorbed and are resorbed by different mechanisms, however, always with the purpose of nutriment uptake. Structurally related compounds which do not serve for nutrition as well as other foreign substances, which have relevant physicochemical properties, are also partially resorbed, i.e., secondary plant ingredients which are not used as primary nutrients, are more or less poorly resorbed by the body.

Since the bioavailability is a prerequisite for a physiological or pharmacological effect, an increase in the bioavailability of the secondary plant ingredients is desirable and is the object underlying the present invention.

This object underlying the present invention is solved by the composition according to claim 1, a product according to claim 6, the use according to claim 9 and the method according to claim 10.

It is known that apolar compounds such as carotene or lycopene are resorbed in a better way in case of a high-fat meal compared to a low-fat meal. It has now surprisingly been found that also polar compounds such as flavones are resorbed in a better way upon simultaneous administration of fat. Particularly, the use of omega-3 fatty acids has turned out to be advantageous. Furthermore, it was unexpectedly observed that sensitive plant ingredients are stabilized in a mixture with said oils, i.e., their degradation does not occur or occurs at a decreased rate.

Suitable oils having a content of omega-3 fatty acids are, for example, borage oil, evening primrose seed oil, currant seed oil, fish oil, linseed oil or perilla seed oil.

Compositions containing one of the following combinations are preferred: extract from Opuntia ficus-indica and perilla seed oil, extract from Vitis vinifera and perilla seed oil, extract from Humulus lupulus and linseed oil, extract from Ginkgo biloba and linseed oil, extract from Crataegus oxyacantha and borage oil, extract from Brassica nigra and borage oil, extract from Echinacea purpurea and evening primrose seed oil, extract from Allium cepa and evening primrose seed oil, extract from Hypericum perforatum and fish oil, extract form Camellia sinensis and fish oil, extract from Glycine max and currant seed oil, extract from Panax ginseng and currant seed oil, extract from Silybum marianum and perilla seed oil, extract from Vitex agnus castus and perilla seed oil, extract from Vaccinium myrtillus and perilla seed oil, extract from Trifolium pratense and perilla seed oil, extract from Myrtus communis and perilla seed oil, extract from Mentha piperita and perilla seed oil,

extract from Linum usitatissimum and perilla seed oil as well as extract from Cimicifuga racemosa and perilla seed oil.

The extracts can be obtained according to per se known preparation methods in variable composition using solvents such as water, methanol, ethanol, 2-propanol, acetone and the like as well as mixtures thereof, at temperatures from room temperature to 100°C under slight to vigorous mixing or by percolation within 10 minutes to 24 hours under normal pressure or elevated pressure. In order to enrich the active ingredients further concentration steps such as liquid-liquid distribution using for example 1-butanol/water or ethylacetate/water, adsorption-desorption on ion exchangers, LH20, HP20 and other resins or chromatographic separations on RP18, silica gel and the like can be performed. The further processing to yield dry extracts is carried out according to per se known methods by removing the solvent at elevated temperature and/or reduced pressure.

To produce administration forms for oral administration a plant extract is mixed with an omega-3 fatty acid-containing oil and filled into capsules, optionally under addition of adjuvants such as stabilizers, fillers and the like.

Examples:

The plant extract is mixed with the oil (both according to the following table) and the flowable suspension obtained is filled into capsules according to a per se known suitable method.

Example 1

	Ingredients	mg/filling of the capsule
1	Extract from red wine (Vitis vinifera)	100.0
2	Perilla seed oil	450.0

Example 2

	Ingredients	mg/filling of the capsule
1	Extract from hop flowers (Humulus lupulus)	100.0
2	Linseed oil	450.0

Example 3

	Ingredients	mg/filling of the capsule
1	Extract from Ginkgo biloba	100.0
2	Linseed oil	450.0

Example 4

	Ingredients	mg/filling of the capsule
1	Extract from Opuntia ficus-indica	100.0
2	Perilla seed oil	450.0

The bioavailability of the flavonoids contained in the plant extracts according to Examples 1 to 4 is increased in the preparations according to Examples 1 to 4 compared to capsules containing the respective plant extract only and not the omega-3 fatty acid-containing oil.

Example 5

300 mg/kg dry extract from blossoms of Opuntia ficus-indica (total extract produced by extraction using 60% by weight ethanol at 50°C to 60°C and subsequent filtration and drying) suspended in 0.2% agar were administered orally by gavage

to male rats (Sprague Dawley). After 2, 4, 8, 24, 30 and 48 h six animals each were killed and plasma was recovered. 200 mg/kg perilla seed oil and immediately after that 300 mg/kg of the extract from Opuntia specified above were administered to another group in the same manner.

The plasma samples were extracted using t-butyl methyl ether (TBME) after enzymatic cleavage by glucuronidase and the flavanol quercetin was determined by HPLC.

Preparation of the samples: 800 µl plasma, 30 µl vitamin C (0.5% in water), 80 µl acetic acid (0.5 M) and 100 µl glucuronidase from Helix pomatia (2000 units glucuronidase; dissolved in water) in 600 µl acetone were held at 37 °C for 1 h and subsequently extracted using 4 ml TBME. The organic solvent was evaporated and the residue was dissolved in HPLC eluent (40% methanol/60% water/phosphoric acid 85 % (pH 2)).

HPLC conditions:

Column:	RP18, Kromasil, 125 x 4 mm
Eluent:	A: water / phosphoric acid 85 % (pH 2)
	B: methanol
Gradient:	0 to 1 min: 60 % A and 40 % B
	1 to 20 min: content of B is increased from 40 to 55 %
Detection:	UV (370 nm)
Flow:	1 ml/min

Result

The quercetin plasma levels are summarized in Table 1.

Time (h)	Quercetin (ng/ml)	
	Extract from Opuntia with perilla seed oil	Extract from Opuntia without perilla seed oil
2	251	159
4	317	239
8	116	94
24	66	33
30	47	33
48	44	27

The plasma levels of quercetin, one of the most common flavanoids, are higher in case of a simultaneous administration of perilla seed oil. The area under the curve ($AUC_{(0-48h)}$) amounts to 4,290 ng/ml.h after administration of extract from Opuntia and perilla seed oil and only to 2,973 ng/ml.h after administration of extract from Opuntia without perilla seed oil. Thus, the bioavailability of quercetin after administration of extract from Opuntia and perilla seed oil is increased by 44% compared to the administration without perilla seed oil.